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In Vivo Studies of Suspension-Feeding Processes in the Eastern Oyster, *Crassostrea virginica* (Gmelin)

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Abstract. Suspension-feeding processes in the eastern oyster *Crassostrea virginica* (Gmelin, 1791) were examined, *in vivo*, with an endoscope linked to a video image-analysis system. We found that many of the previously published concepts of particle transport and processing in this species, obtained using surgically altered specimens or isolated organs, are incomplete or inaccurate. In particular, our observations demonstrate that (1) captured particles are transported along the gills by both mucociliary (marginal grooves) and hydrodynamic (basal tracts) processes; (2) the labial palps accept material from the gills both in mucus-bound particle strings (transported in marginal grooves), and suspended in particle slurries (transported in basal tracts); (3) the labial palps reduce the cohesive integrity of the mucous strings and disperse and sort the entrapped particles; (4) particles are ingested in the form of a slurry; and (5) ciliary activity on the labial palps is independent of that on the lips, allowing the oyster to filter particles from suspension and produce pseudofeces without ingesting any particulate matter. Because many ostreids have the same plicate gill structure, we believe that our conclusions are applicable to other oyster species. In addition, the present observations are consistent with other endoscopic examinations recently made on bivalves in different families. We conclude that accepted theories of particle handling in suspension-feeding bivalve mollusks must be modified to accommodate observations made with the endoscope.

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Abbreviations: OIT—optical insertion tube of the endoscope.

Introduction

The eastern oyster *Crassostrea virginica* (Ostreidae, Bivalvia) is an ecologically important species, often forming dominant epibenthic populations on the Atlantic and Gulf Coasts of North America. These oyster populations can influence the surrounding environment through particle depletion, nutrient cycling, and biodeposition (Dame *et al.*, 1984; Jordan, 1987; Newell, 1988). In addition, *C. virginica* forms the basis of a commercially important fishery throughout its range. As a consequence of their ecological and economic value, eastern oysters have been studied extensively, and numerous reports about their ecology, physiology, and anatomy have been published (for reviews see Galtsoff, 1964; Eble *et al.*, 1994). Specifically, the capture of particles on the gills; transport of particulate matter to the labial palps, mouth, and stomach; and processing of food material in the alimentary system have been thoroughly described (*e.g.*, Menzel, 1955; Nelson, 1960; Galtsoff, 1964; Ribelin and Collier, 1977; Newell and Langdon, 1994; Langdon and Newell, 1994).

Unfortunately, methodological limitations have constrained the study of feeding processes in whole, intact oysters. Nelson (1923) and Menzel (1955) did describe some aspects of particle capture and transport in whole juvenile oysters; their light microscopical observations were made through the transparent shells of post-set specimens that had been allowed to metamorphose and grow attached to glass microscope slides. With these exceptions, however, most reports about particle capture and transport by the pallial organs have been based on observations of isolated structures, or examinations of structures in sur-

gically altered oysters (*e.g.*, Nelson, 1960; Galtsoff, 1964). Although such studies underlie our present understanding of suspension-feeding in most bivalve families, including the Ostreidae, problems inherent in the observational techniques may have led to erroneous or incomplete conclusions. For example, removal and isolation of the gills destroys the subtle hydrodynamic interactions that often exist between these structures and moving particles (Beninger *et al.*, 1992; Ward *et al.*, 1993), and perturbs the physiological and neurological mechanisms that control muscular and ciliary movement. Surgery can also alter the normal flow of water through the pallial cavity and may damage delicate feeding structures. Furthermore, surgery can stimulate excess mucus production and cause the feeding structures to function abnormally (Jorgensen, 1976).

In contrast, recently developed techniques in video endoscopy (Ward *et al.*, 1991) have allowed us to reevaluate suspension-feeding mechanisms in whole, intact bivalves (Beninger *et al.*, 1992; Ward *et al.*, 1993). There are many advantages of video endoscopy over previous techniques: (1) no surgical alteration of tissue is required; (2) the optical insertion tube (OIT) of the endoscope is small enough (1.7 mm diameter) to be inserted between demibranchs of the gill, between opposing labial palps, and even into the alimentary canal; and (3) video recording and image analysis facilitate observations and their documentation, and permit post-observational analysis of the biodynamics of particle processing.

In this study, we employed video endoscopy to examine, *in vivo*, the feeding structures and mechanisms in *C. virginica*. Particle kinematics were studied, from the point of capture on the gills, to post-ingestive processing in the stomach. Of particular interest were the modes of particle transport (*e.g.*, mucous-bound or suspended), and their implications for particle sorting and ingestion. We then used our results to address several fundamental questions about suspension feeding in intact oysters: (1) What are the mechanisms of particle sorting on the gills? (2) What is the mode of particle transport on the frontal surfaces and margins of the gills? (3) How are particles transferred from the gills to the labial palps? (4) what are the mechanisms of particle sorting and transport on the labial palps? (5) In what mode are particles ingested? (6) Are particles in the stomach tightly bound in mucus, or freely suspended? Finally, we compared our endoscopic observations of intact oysters with previous reports of feeding processes in order to better understand suspension-feeding mechanisms in the Ostreidae.

Materials and Methods

Ten *Crassostrea virginica* adults were maintained in an aerated 60-l aquarium at the Marine Sciences Research Laboratory, Memorial University of Newfoundland. Seawater in the container was replaced every other day, and was maintained at 15–21‰ and 15–20°C. Oysters were fed a daily maintenance ration of the cultured diatom *Chaetoceros muelleri* Lemmermann.

Specimens were prepared for endoscopy by carefully trimming a small section (about 3–6 cm in length × 0.5–1 cm in width) of the inhalant margin of the upper and lower valves. This was done without damaging the underlying mantle margins and produced a narrow opening in the shell. Trimming of the shell served three purposes. First, it allowed us to introduce the optical insertion tube (OIT) into the pallial cavity of an oyster, even when the specimen was closed and not feeding; second, it provided more freedom of movement for the OIT when the specimen was open and actively feeding; third, it prevented the shell edges from damaging the OIT when the specimen adducted its valves. Oysters prepared in this way were allowed to recover for at least one day before use; often shell repair began during the several weeks that these animals were held in the aquarium.

Several specimens were further manipulated so we could insert the OIT through the mouth and into the alimentary canal. Because *C. virginica* is monomyarian, the anterior portion of the body is not attached to the shell by muscles and could be carefully lifted through the narrow opening we had cut in the shell. The body was gently held in an extended position with several monofilament nylon lines attached to hooked retractors that were inserted into the outer portions of the visceral mass. This fully exposed the mouth and allowed us to insert the OIT. A similar procedure was used by investigators wishing to inject latex (Galtsoff, 1964) or food material (Newell and Langdon, 1986) directly into the oyster's alimentary system.

Endoscopy was performed according to methods described by Ward *et al.* (1991). Briefly, the endoscope (OIT = 1.7 mm in diameter) was connected to an optical zoom-adapter and attached to a monochrome, charge-coupled-device camera. The resolution of the video-endoscope was about 5 μm at a maximum magnification of about 150 ×. Video signals were recorded on an 8 mm VCR (Hi8). The recorded images were then digitized with a video digitizing board (RasterOps, Corp.) and enhanced for morphometric analysis with Adobe Photoshop software (Adobe Systems, Inc.).

Morphometric measurements made on the digitized images were calibrated by isolating the pallial organs (*e.g.*, gills, labial palps, lips) of several specimens, and measuring the width of various structures (*e.g.*, filaments, plicae, ciliated ridges) with a compound microscope and a calibrated ocular micrometer. Particle velocities could then be determined by counting the number of frames required

for a particle to traverse a known distance along a given organ; recording speed was 30 frames $\cdot s^{-1}$ (NTSC format). Velocities ($\mu m \cdot s^{-1}$) are presented as means $\pm$ 1 standard deviation.

During endoscopic examination, the oysters were held in an aerated, closed seawater system at the same temperature and salinity as the holding aquarium. The specimens were allowed to feed freely on natural seston supplemented with various particles, including silica (2–6 μm diameter), reflective red plastic particles (about 5 μm diameter, Radiant Color, Hercules Inc.), polystyrene microspheres (about 18 μm diameter, Polysciences Inc.), spray-dried *Tetraselmis* sp. (2–13 μm diameter, Celsys, Cell Systems Ltd.), and the diatom *C. muelleri* (about 6 μm diameter, cultured in f/2 medium; Guillard, 1975). Prior to use, *Tetraselmis* cells were pelleted twice in a centrifuge and resuspended in 1 liter of seawater; they were then resuspended by sonication at low power for 10 min. The polystyrene microspheres were cleaned with 30% H_2O_2 for 5 min and washed with 1 liter of filtered seawater or distilled water; they were then sonicated for 10 min. To increase particle concentration above the background level (10^4 particles $\cdot$ ml^{-1}), we delivered 2–5 ml of stock particle suspensions (10^5–10^6 particles $\cdot$ ml^{-1}), as needed, to the inhalant margin of oysters using a peristaltic pump or Pasteur pipet.

Whole cell extracts prepared from *C. muelleri* cultures were used to test the effects of chemical stimulation on feeding and particle selection. Diatom cells were pelleted in a centrifuge, frozen at −20°C, thawed, and sonicated for 30 min. The disrupted cells were then passed through a GF/A glass fiber filter, and the filtrate collected and stored at −20°C until used (Ward *et al.*, 1992). In some experiments, microspheres were treated with the algal extract. This was done by activating the spheres with 100 ml of 100% methyl alcohol and then treating them with 50 ml *C. muelleri* whole cell extract (Ward, 1989).

Results are based on the endoscopic examination of ten oysters, and are presented as a composite of these observations. In some instances, surgically altered specimens were observed with a dissecting microscope, recreating the conditions under which previous workers had obtained their results. Finally, to verify certain structural aspects of the anterior portion of the demibranchs, scanning electron microscopy (SEM) was performed on isolated gill segments according to standard procedures (Glauert, 1980; Bozzda and Russell, 1992).

The anatomy of ostreid gills is often described in terms that vary from one publication to another. This is because the gills of oysters arch around the adductor muscle to such a degree that, depending on their location, portions of the demibranchs can occupy a posteroventral, ventral, or anterior position. In this publication we use the terminology of Atkins (1937b) and Nelson (1960). The ciliated regions that lie along the attached edges of the gills are herein termed basal ciliated tracts. Synonyms for these tracts include the dorsal (Nelson, 1923; Menzel, 1955), proximal (Nelson, 1960), or axial (Yonge, 1926) tracts, grooves, or furrows. The ciliated grooves that lie along the free edges of the gills are herein termed marginal ciliated grooves. Synonyms for these grooves include the ventral (Nelson, 1923; Menzel, 1955), lower-marginal or free-marginal (Yonge, 1926), and terminal (Galtsoff, 1964; Bernard, 1974) grooves or furrows.

All anatomical drawings have the dorsal-ventral axis oriented so that ventral is at the top and dorsal is at the bottom of the sketch. Although this orientation does not adhere strictly to convention, it does match the perspective depicted in the endoscope micrographs. This circumstance arose because the OIT was always inserted through the inhalant margin of the shell, so that the free margins of the pallial structures were the first to come into view. Furthermore, this orientation more closely reflects the position of oysters in nature.

Results

For clarity, our observations of suspension feeding in *Crassostrea virginica* are grouped into two sections. In the first section we deal with the anatomy and function of the pallial organs and alimentary canal, with emphasis on our novel observations. In the second section we deal with the functional importance of these observations to suspension-feeding processes, and describe the capture and transport of particles by the feeding organs described in the first section. We do not recapitulate the detailed anatomy of these organs; rather, we present novel observations of organ movements and functions and describe particle transport from gills to stomach. Where appropriate, the results are placed in the context of previous descriptions of feeding in oysters.

A. Organ structure and function

1. Gills. The most conspicuous structures in the pallial cavity of the oyster are the deeply plicated, heterorhabdic gills, which have been described in detail previously (Fig. 1A) (Atkins, 1937c; Nelson, 1960; Galtsoff, 1964). With the endoscope we could clearly see the ordinary filaments and the interfilamentary spaces that lead to the ostia (Fig. 1B), as well as the metachronal waves produced by cilia.

Our observations of the gills by means of endoscopy, light microscopy, and SEM reveal that modifications of previously reported descriptions must be made. The marginal ciliated groove does not extend along the entire length of each demibranch. Instead, at the anterior portion adjacent to the palps, the groove becomes narrower and

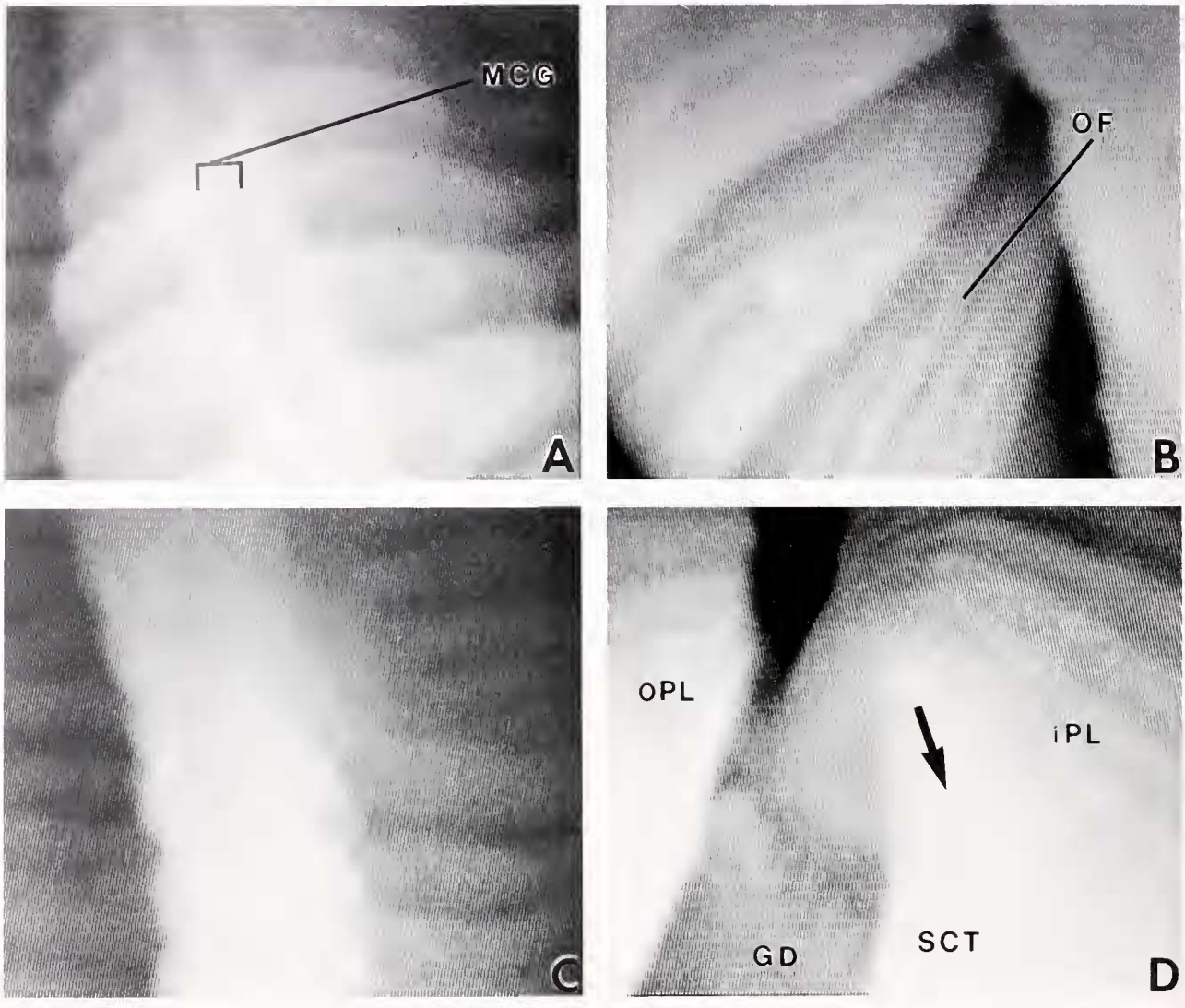


Figure 1. Video-endoscope micrographs showing, *in vivo*, several pallial structures of *Crassostrea virginica* (A) The margin of one gill demibranch is shown in normal feeding position. The deeply plicated structure of the demibranch can be seen. The ordinary filaments appear as lines running parallel to the axis of each plica (length of gill section = about 1.6 mm). (B) At higher magnification the ordinary filaments of the plicae are visible, as are the openings to the interfilamentary spaces that lead to the ostia, which appear as dark slits between the filaments (width of each filament = about 35 μm). (C) The margin of the gill demibranch displayed in A shown seconds later after contraction of the plicae. (D) A view of the anterior termination of one demibranch and the basal region of the surrounding labial palps. The ridged surfaces of opposing palp lamellae are visible. Note the smooth ciliated tract that lies at the posterior edge of each lamella and carries particles (*e.g.*, white dot) from the base of the palp towards the distal margin (arrow; width of each palp ridge = about 60 μm). (GD = gill demibranch, MCG = marginal ciliated groove, OF = ordinary filament, iPL = inner palp lamella, oPL = outer palp lamella, SCT = smooth ciliated tract).

shallower, resembling an indentation that runs along the midline of the margin (Fig. 2A, B). This indentation ends 2–3 plicae from the anterior termination of each demibranch (Fig. 3A). The transition from groove to indentation begins where the demibranch curves sharply towards its attached base on the visceral mass, about 7–10 plicae from the anterior termination of the demibranch (Figs. 2B; 3A). We call this anatomical location the "inflection point." It exists because the line of fusion of the ascending and descending lamellae is being distorted by the sharp decrease in height of each successive filament. The most anterior plica of each demibranch is composed

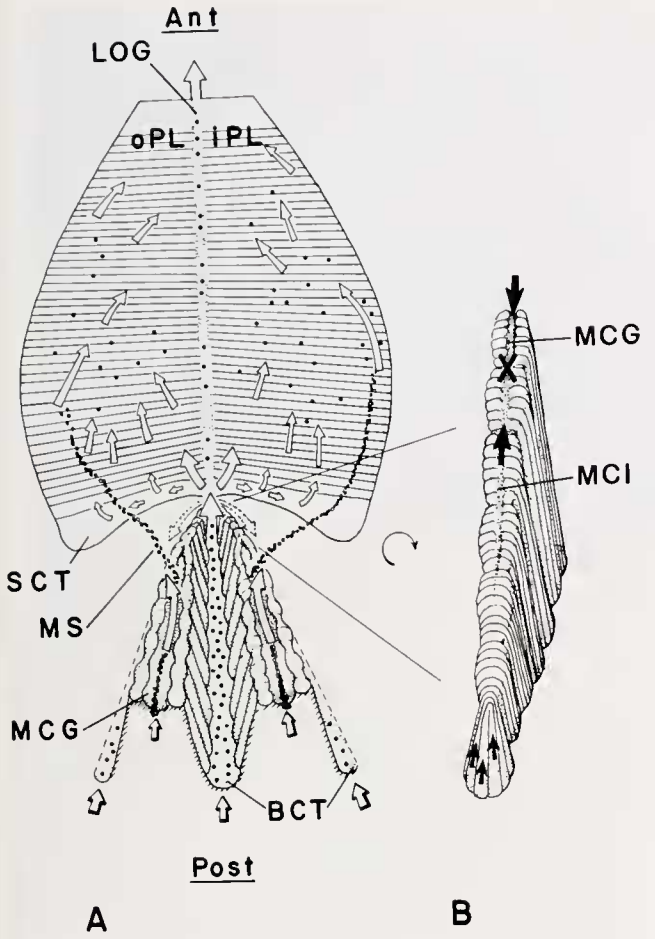


Figure 2. (A) Schematic diagram of the anterior portion of two demibranchs and their junction with one pair of labial palps of *Crassostrea virginica*. The palps are drawn folded back to reveal the direction of net particle movement (arrows) on the ciliated tracts and the ridged surfaces. Note that the cohesive integrity of mucous strings from the marginal ciliated grooves is reduced by the action of the palps so that the entrapped particles disperse. After sorting, particles are transported to the lips (smooth region at anterior edge of palp) and then mouth (not shown) for ingestion. (B) An enlargement of the anterior termination of one demibranch, which has been rotated 180° about its anterior-posterior axis. Note that the marginal groove becomes narrower and shallower 7–10 plicae from the termination. The transition from groove to indentation occurs at the inflection point of each demibranch (marked by X). Cilia in the groove and indentation beat in opposite directions (large arrows), and mucous balls form at the junction of these oppositely beating ciliary tracts (X). Excess particles from the basal gill-palp junction are transported to the marginal ciliated indentation (small arrows) via the ordinary filaments. (Ant = anterior, BCT = basal ciliated tract, LOG = lateral oral groove, MCG = marginal ciliated groove, MS = mucous string, iPL = inner palp lamella, oPL = outer palp lamella, Post = posterior, SCT = smooth ciliated tract).

entirely of ordinary filaments and wraps around the end of the demibranch, forming a rounded anterior termination (Fig. 2B). Along the entire anterior margin of each demibranch is a broad ciliated tract that covers the marginal indentation and transports material posteriorly towards the inflection point (Figs. 2B; 3B).

Our observations of gill movements within an intact oyster confirm previous reports made by Menzel (1955), Nelson (1960), and Galtsoff (1964). These findings include changes in the position of the demibranchs, and the occasional contraction and expansion of the plicae caused by the lateral movements of the ordinary filaments (Fig. 1A, C). Such movements of the gills probably force hemolymph into interfilamentary and interlamellar tissues (Elsey, 1935; Menzel, 1955; Galtsoff, 1964) and may facilitate the movement of water through the water tubes and into the epibranchial chamber.

2. Labial palps. The labial palps are positioned at the anterior termination of the gills. Endoscopic observations on the morphology of the palps confirm previous descriptions (Menzel, 1955; Nelson, 1960; Galtsoff, 1964), with only one modification. Along the entire free edge of each palp, nearest the gills, is a smooth, ciliated tract, two to three palp ridges wide. This tract, previously unreported, transports particles from the base of the palps to the apex (Figs. 1D; 2A).

The labial palps of oysters exhibited changes in muscular and ciliary activity that were positively correlated with increased particle capture and transport. When the particle concentration was low (10^4 particles $\cdot$ ml^{-1}), activity was also low. As concentration increased, more particles were transported anteriorly, and muscular and ciliary activity of the palps also increased.

During periods of active particle clearance, the palps constantly interacted with the gills. The apices of the palps would move along their adjacent demibranchs, from the ascending lamellae, across the marginal ciliated groove, to the descending lamellae, or *vice versa*. When this occurred, the inner and outer lamellae of one palp pair alternated between enclosing one and two demibranchs of a gill. The palps were often observed surrounding only one demibranch, but this condition did not interfere with the transfer of mucous strings from the gill to palp (see section B2). When actively removing material from the gills, the lamellae of each palp pair moved independently and alternated between being spread slightly apart and being closely appressed (Fig. 4A, B). When apart, some particles remained in close proximity to the ridged surfaces, while others occupied the space between the two opposing surfaces. The significance of this condition will be explained in section B2.

3. Lips and buccal region. The outer and inner lips of the oyster arise from the most anterior edge of the two outer and two inner palp lamellae, respectively (Fig. 5A; see also Galtsoff, 1964). Here, the ridged surfaces of the palps terminate and the smooth surfaces of the lips begin. The lateral walls of the lips possess broad tracts of cilia

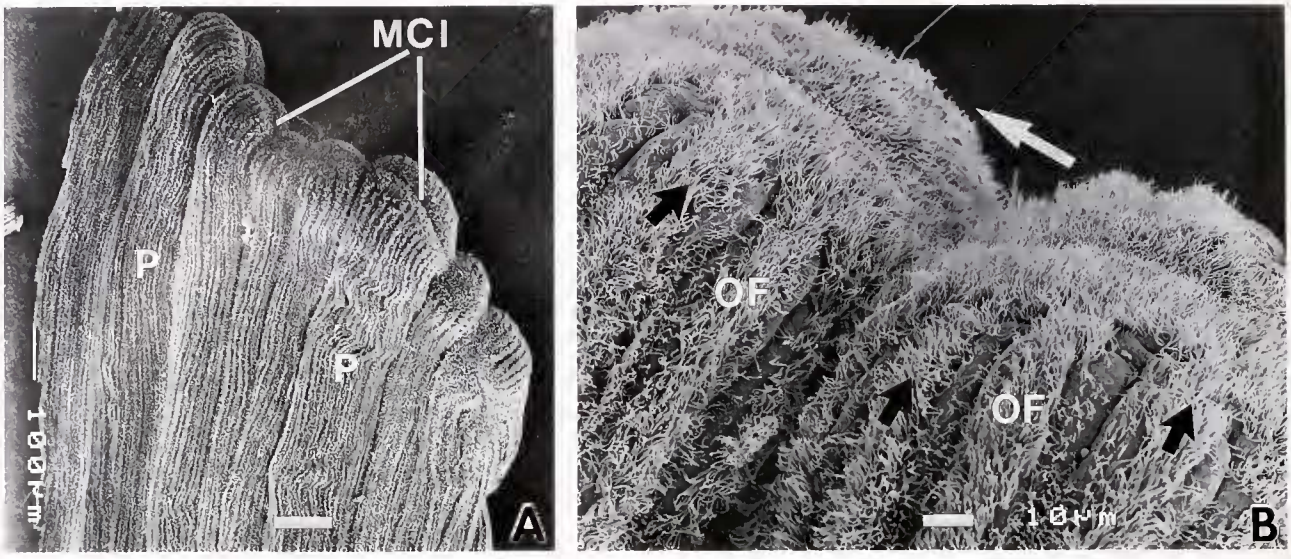


Figure 3. Scanning electron micrographs of the anterior termination of one gill demibranch of *Crassostrea virginica* (A) The distal edges of the seven most anterior plicae are shown, demonstrating that the marginal ciliated groove no longer exists and has formed an indentation that runs along the midline of the demibranch margin. Notice that the most anterior two plicae (far right side) lack this indentation. (B) Close up of the demibranch margin showing the ciliated tract that covers the indentation and directs particles posteriorly (white arrow). Notice the frontal cilia of the ordinary filaments that direct particles onto the marginal indentation (black arrows). Scale bars: A = 100 μm, B = 10 μm. (MCI = marginal ciliated indentation, OF = ordinary filament, P = plica).

that beat towards the mouth. A previously undescribed, highly ciliated tract runs along the entire free margin or ridge of the inner lip. When the inner lip is inserted into the outer lip, this ridge aligns with a ciliated tract on the outer lip (Figs. 4C, D; 5B). Prominent cilia on both the inner lip ridge and outer lip tract beat towards the mouth. A portion of the outer lip tract, just below the insertion of the inner lip, can be drawn in to form a gutter (Fig. 5B). The formation of this gutter usually coincided with active feeding.

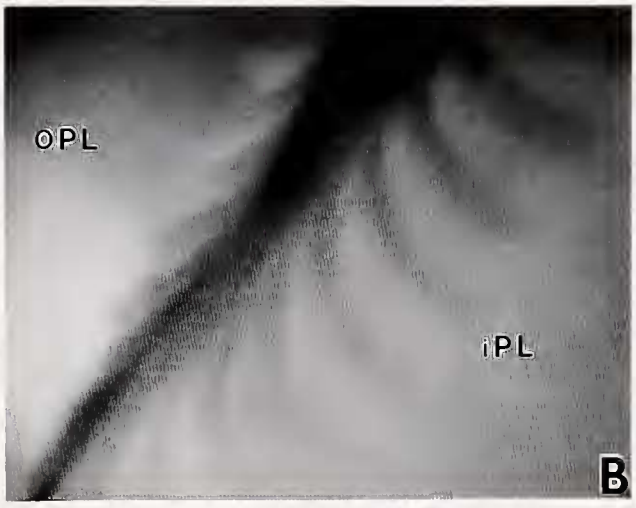
The frequency of ciliary beating on the lips was not constant, nor was it associated solely with changes in particle capture and transport. On occasion, ciliary activity abruptly ceased and then, after a period of time, restarted. Furthermore, ciliary activity on the lips was independent of that on the palp ridges. Oysters that were feeding at background particle concentrations often had active gill and palp cilia but little or no ciliary activity on the lips. In one such specimen, several milliliters of *Chaetoceros muelleri* culture (10^6 cells · ml^{-1}) were introduced into the pallial cavity. Over a 5-min period, ciliary activity on the inner lip ridge and outer lip tract gradually increased until all cilia were actively beating.

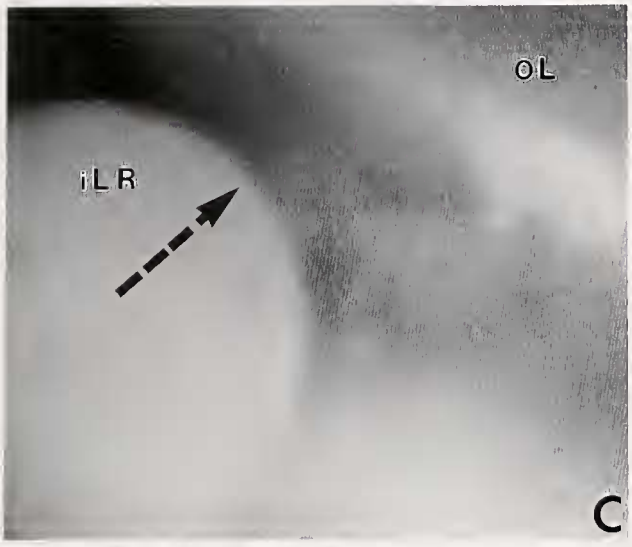
In dissected oysters, cilia on the lips were either inactive or exhibited an irregular activity. In contrast, cilia on the gills and palps continued beating for many hours after dissection.

4. Esophagus and stomach. Our observations of the internal, gross morphology of the alimentary canal of an intact oyster confirm previous reports made by Owen (1955) and Galtsoff (1964). One modification of these reports, however, is that the stomach is not a chamber or cavity, but rather a compressed structure with walls that are tightly appressed. This is a consequence of the typhlosole protruding into the food-sorting caecum. The intestinal groove that carries rejected particles from the caecum is actually deep and concealed, hence preventing particles from reentering the main portion of the stomach (Purchon, 1977; Langdon and Newell, 1994). The conspicuous crystalline-style sac is lined with strong ridges that support tracts of actively beating cilia (Fig. 4E). The cilia rotated the style in a clockwise direction. To our knowledge, this study is the first to document crystalline style activity in an undissected bivalve.

B. Particle capture and transport

1. Gills. Particles entrained in the inhalant water current were first carried dorsally in the pallial cavity and then deflected laterally towards the frontal surfaces of the gill lamellae. Often, particles were drawn directly into the "troughs" between adjacent plicae, presumably by currents produced by the lateral cilia of the principal and transitional filaments that form each trough (Fig. 6; Nel-





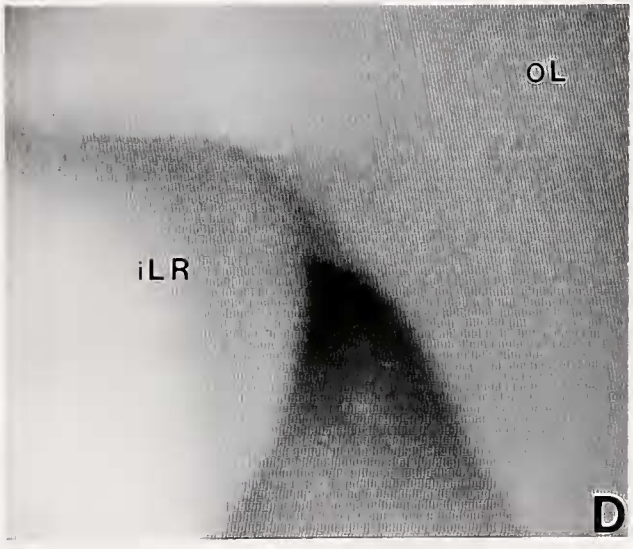


Figure 4. Video-endoscope micrographs showing, *in vivo*, several pallial and internal structures of *Crassostrea virginica*. (A) The distal margin of one pair of labial palps is shown with the lamellae closely appressed and (B) slightly apart. In B, note the ridged surfaces of the palps and the deep rejection tracts between each ridge (length of palp sections = about 600 μm). (C) In the buccal region, the highly ciliated inner lip ridge and outer lip are shown before and (D) after insertion of the inner lip into the outer (dashed arrow indicates inner lip movement; width of inner lip ridge = about 500 μm). (E) In the pyloric caecum of the stomach, the opening to the crystalline style sac is shown. Note the ciliated ridges that line the sac and rotate the crystalline style (not visible under our lighting conditions). Protrusion of one typhlosole can also be seen (diameter of sac = about 1.5 mm). (iLR = inner lip ridge, oL = outer lip, iPL = inner palp lamella, oPL = outer palp lamella, T = typhlosole).

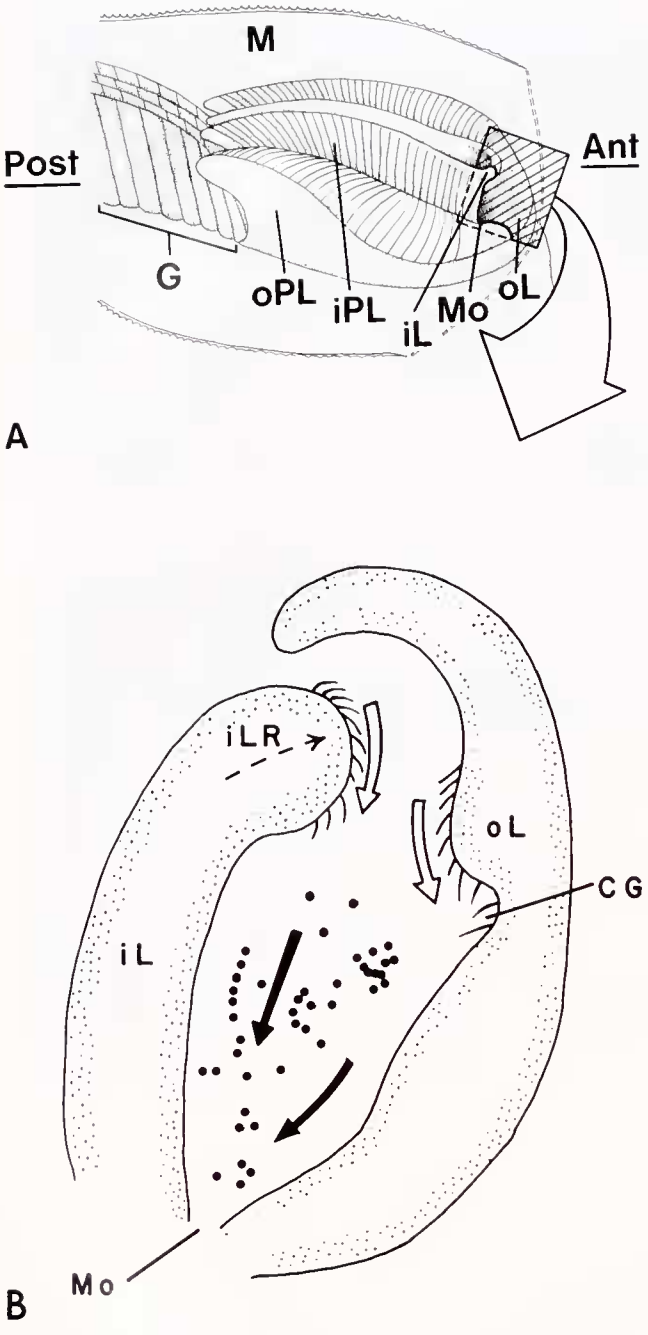


Figure 5. (A) Schematic diagram of the lips, labial palps, and anterior portion of the gills of *Crassostrea virginica* (redrawn after Galtsoff, 1964). The oral hood of the mantle is cut away to reveal the buccal region section shown in B. (B) An enlargement of the mid-longitudinal section of the buccal region. Prominent cilia on the inner lip ridge and outer lip tract beat orally (open arrows), and aid in the transport of particles into the mouth (solid arrows). Buccal region is shown before insertion of the inner lip ridge into the outer lip (dashed arrow indicates inner lip movement). Outer lip is shown with ciliated gutter formed which coincides with active feeding. (Ant = anterior, CG = ciliated gutter, G = gills, iL = inner lip, iLR = inner lip ridge, oL = outer lip, M = mantle, Mo = mouth, iPL = inner palp lamella, oPL = outer palp lamella, Post = posterior).

son, 1960; Galtsoff, 1964; Ribelin and Collier, 1977). Once in the plical troughs, particulate matter was directed basally, at a mean velocity of 740 ± 250 $\mu m \cdot s^{-1}$, towards the ciliated tracts that lie at the base of the gills (Table I; Fig. 6). Particles in the troughs appeared to be transported in suspension, but the high variation in particle velocity (Table I) suggests that some particles may have been carried directly on the frontal cilia of the surrounding transitional filaments.

Particles were also captured on the frontal surface of the ordinary filaments. The ordinary filaments form the sides and tops of the plicae, from the transitional filament on one side, to the transitional filament on the other, an area we term the plical "crest." Capture of particles by the ordinary filaments was not always instantaneous. Particles were often observed being deflected away from the frontal surface of one filament, only to accelerate towards the frontal surface of the same or an adjacent filament. These particles appeared to be "bouncing" from one ordinary filament to another on the crests of the plicae. Such particles usually moved perpendicularly to the long axis of the gill filaments and often moved into the plical troughs (Fig. 6).

Most particles captured on the ordinary filaments were directed—presumably by the coarse frontal cirri (Ribelin and Collier, 1977)—towards the marginal ciliated grooves that lie at the distal edges of the demibranchs. Occasionally, particles on the ordinary filaments were moved basally for a short distance—presumably by the fine frontal cilia (Ribelin and Collier, 1977)—before being transferred onto the tracts of the coarse frontal cirri and directed marginally (Fig. 6). On any ordinary filament, particles progressed uniformly at a mean velocity of 120 ± 30 $\mu m \cdot s^{-1}$ (Table I). Particles moved towards the marginal grooves in an orderly procession, with trailing particles never overtaking leading ones. The distance between a given particle and the underlying filament could not be estimated, because the separation was smaller than the resolution of the endoscope. But no particles were observed being transported in water currents, 20–25 μm above the filament surface, as proposed by Jørgensen (1975, 1981) for the mussel *Mytilus edulis*.

The manner in which large and small particles (18 μm and 5 μm diameter) were transported along the frontal surfaces of the ordinary filaments was similar. When oysters were provided only with small plastic particles, most of these were taken to the marginal ciliated grooves. When mixed with similar-sized *Chaetoceros muelleri* cells, more plastic particles were observed in the basal ciliated tracts. Polystyrene microspheres treated with diatom extract were transported to the basal tracts and marginal grooves in the same manner as untreated microspheres. We observed

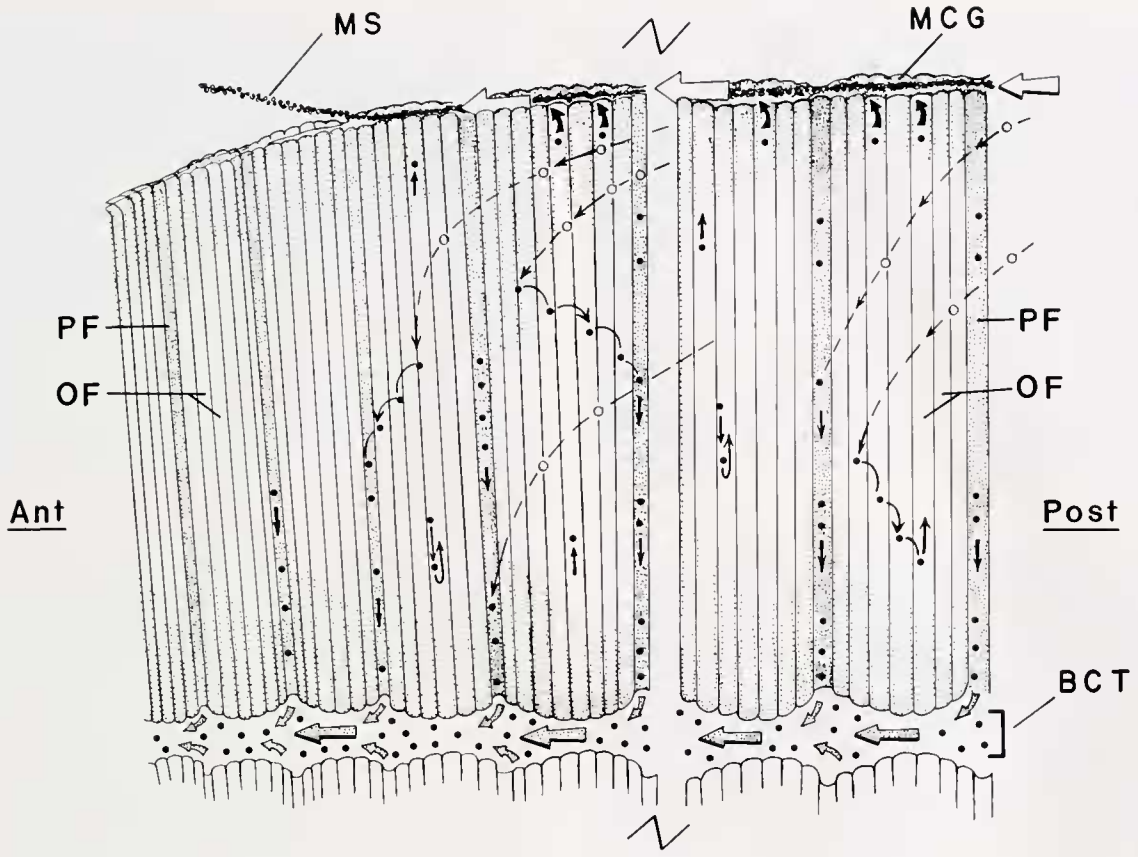


Figure 6. Schematic diagram of typical sections of one gill demibranch of *Crassostrea virginica* showing particle capture and transport on the lamella. Dashed lines indicate the path of particles before being captured by the gills (open circles), and solid lines indicate movement of particles after they contact the gill surface (solid circles). Note that after capture, some particles on the ordinary filaments reverse direction as they are passed from the fine frontal to the coarse frontal ciliated tracts. Other particles "bounce" from one ordinary filament to another and are usually deflected into the plical troughs. Note that a principal filament lies at the base of each plical trough. Particles in the basal tracts are transported anteriorly suspended in a slurry (stippled arrows), while those in the marginal grooves are transported anteriorly in a mucous string (open arrows). (Ant = anterior, BCT = basal ciliated tract, MCG = marginal ciliated groove, MS = mucous string, OF = ordinary filament, PF = principal filament, Post = posterior).

no obvious difference in the quantity of spheres that entered the tracts and grooves, regardless of treatment.

Particles that left the plical troughs and entered the basal ciliated tracts were immediately swept anteriorly in suspension (Figs. 6; 7A). No particles were observed entering these tracts from the ordinary filaments. Both individual particles and loose particle aggregations were observed being carried in cilia-generated currents within the basal tracts. When oysters were exposed to a higher particle concentration, individual particles became more numerous, aggregations became larger, and the material in the ciliated tracts took on the appearance of a slurry. Particles in this slurry moved anteriorly at a mean velocity of 820 $\pm$ 240 $\mu m \cdot s^{-1}$ (Table 1) and flowed around the base of each plica, conforming to the shape of the tract. Particles and clumps in the basal tracts frequently changed position relative to one another, and trailing particles often overtook leading ones. The movement of suspended material in the basal ciliated tracts contrasted with that in the marginal ciliated grooves (see below).

Particles that were transported to the distal edges of the ordinary filaments were carried into the marginal grooves by the coarse, terminal cirri. These cirri beat obliquely toward the palps (Atkins, 1937b), causing particles that were entering the grooves to move in an arc directed anteriorly (Fig. 6). In each marginal groove, particles were immediately incorporated into a continuous, cohesive mucous string (Figs. 6; 7B). Mucous strings were always observed inside the well-developed grooves, even when few particles were being captured and transported to the

Table I

Summary of direct observations of particle transport on the gills of the oyster, Crassostrea virginica

Location	Mode of particle transport	Mean velocity ± SD ($\mu m\ s^{-1}$)	Range ($\mu m\ s^{-1}$)	n
Frontal surface				
Plical trough	Hydrodynamic	740 ± 250	366–1225	11
Plical crest	Mucociliary	120 ± 30	70–163	10
Basal ciliated tract	Hydrodynamic	820 ± 240	462–1225	15
Marginal ciliated groove	Mucociliary	280 ± 30	245–318	10

Note that rate of transport in the plical troughs and basal ciliated tracts is about 3–5 times higher than that on the plical crests or in the marginal ciliated grooves (n = number of measurements made for each mean velocity determination).

grooves. Material in the marginal ciliated grooves moved anteriorly at a mean velocity of 280 ± 30 $\mu m \cdot s^{-1}$ (Table I). Once bound in mucus, particles maintained their relative positions, and trailing particles did not overtake leading ones. Thus, the movement of material in the marginal grooves was similar to that on the frontal surfaces of the ordinary filaments, although particles in the marginal grooves were clearly incorporated in a mucous matrix.

Mucous strings in the marginal ciliated grooves moved anteriorly towards the palps until they reached the termination of the grooves at the inflection points of the demibranchs. Here the material collected into masses that slowly revolved, forming balls of mucus-bound particles (Fig. 8A). These balls continued to grow until they were picked up by the labial palps (see section B2.), or were swept off the margins and onto the mantle by pallial water currents and then rejected as pseudofeces.

Particles that were carried on the ordinary filaments to the demibranch margins anterior to the inflection points, were transported posteriorly on the marginal ciliated indentations. These particles, which were moving in the opposite direction to those in the marginal grooves, were then incorporated into the mucous balls that formed at the inflection points (Figs. 2B; 8A).

2. Labial palps. Particulate matter that left the anterior termination of the basal ciliated tracts entered one of two small spaces between the gills and palps that we call the "basal gill-palp junction." These junctions are roughly triangular with the bases pointing towards, and accepting particles from, the basal tracts (Fig. 7C). In the eastern oyster, there are five basal tracts (two outer, two inner, one medial) and two lateral oral grooves of the palps (one left, one right; Nelson, 1960). Each gill-palp junction (left and right) received particles from at least one outer and one inner basal tract. Particulate matter in the medial tract was directed into either the left or right junction by the labial palps. The direction of flow of particles in the medial tract is controlled by adjustments in the positions of the posterior, proximal edges of the left and right inner palp lamellae.

Particulate matter in each basal gill-palp junction was in the form of a slurry, and individual particles left the junction in one of four directions: (1) into the lateral oral groove of the palps and directly towards the mouth, (2) onto the ridged surfaces of the palp lamellae, (3) onto the smooth, ciliated tract that lies along the posterior edge of each palp lamella and then distally, or (4) onto the marginal ciliated indentation at the anterior termination of each demibranch and then posteriorly (Figs. 2A, B; 7C). The mechanisms responsible for controlling the direction of particles from this junction could not be ascertained with our methods. But the process rapidly redirected particles and loose aggregations that continuously entered the gill-palp junctions at a mean velocity of 820 $\mu m \cdot s^{-1}$.

Under our experimental conditions, most particles entering the basal gill-palp junctions were directed into the lateral grooves or onto the ridged surfaces of the labial palps. Fewer particles were directed onto the smooth, ciliated tracts of the palps. Only at high ambient seston concentrations, which overloaded the gill-palp junctions, were particles directed onto the marginal indentations at the anterior terminations of the demibranchs.

Particles that were directed into the lateral oral grooves could subsequently be ejected onto the ridged sorting surfaces of the palps within the first few millimeters of the start of the grooves (Fig. 7D). Particulate matter that remained in the grooves beyond this location was never ejected, presumably being carried directly to the lips and mouth, and was not subjected to sorting by the palps. Distally moving particles on the smooth, ciliated tracts were usually redirected onto the ridged surfaces of the palp lamellae along the entire length of the tracts, but some particles were carried to the apices of the palps (Figs. 2A; 8A).

As described in section B1, particles in the marginal ciliated grooves of the demibranchs were carried anteriorly in mucous strings until they reached the inflection points where anteriorly directed ciliary action ceased. At these sites, the mucus-bound particle strings began rotating, forming balls as material continued to reach the same location (Fig. 8A). Occasionally, a mucous ball would continue to grow until it was swept off the margin of the demibranch by pallial water currents. More often, the apex of one palp lamella would contact the ball (Fig. 9A) and lift it onto the ridged palp surface. This action established the integrity of a particle string between the demibranch

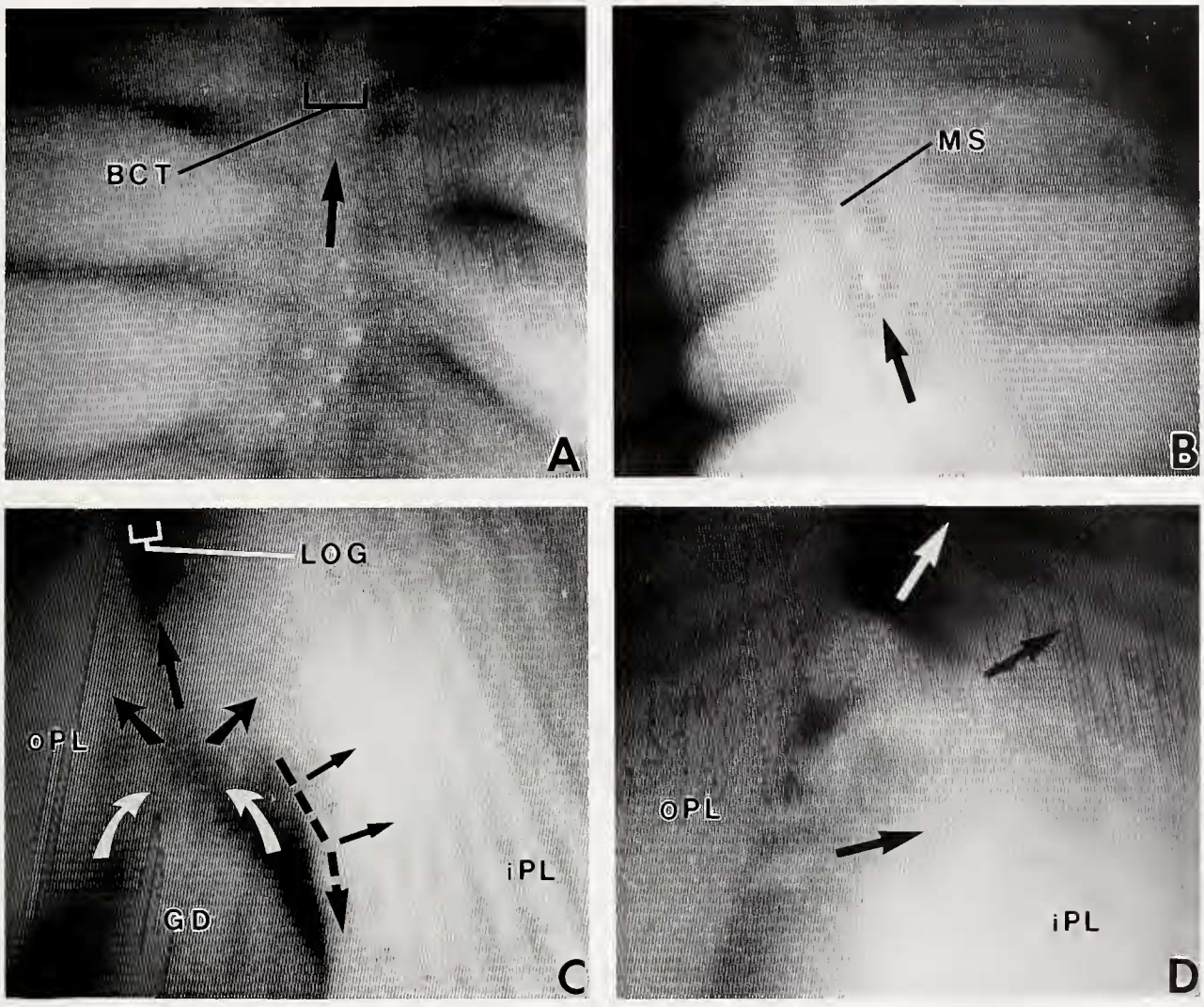


Figure 7. Video-endoscope micrographs showing, *in vivo*, the movement of particles on several pallial structures of *Crassostrea virginica*. Arrows indicate direction of net particle movement. (A) At the base of the gills, the plicae fuse to form the basal ciliated tracts. Particles (white spots) are transported anteriorly in each tract in suspension (length of gill section = about 1.0 mm). (B) At the margin of the gills, particles bound in a mucous string are transported anteriorly in each marginal ciliated groove (length of gill section = about 1.6 mm). (C) At the basal gill-palp junction, particles exit the basal tracts on either side of a gill demibranch (white arrows) and are directed, either anteriorly into the lateral oral groove, or onto the ridged surfaces of the palps (black arrows). Dashed arrow indicates movement of particles along the smooth ciliated tract of the inner palp lamella; these particles are then distributed across the ridged surface. Movement of particles onto the marginal ciliated indentation of the demibranch is not shown (width of each palp ridge = about 60 μm). (D) A particle aggregation being rejected from the lateral oral groove just anterior to the basal gill-palp junction shown in C. The aggregation is being disrupted by the action of the ridged surfaces of the two opposing palp lamellae. Arrows indicate direction of movement of smaller masses which are breaking from the original aggregation (width of each palp ridge = about 60 μm). See Figures 1–4 for descriptions of the form and function of pallial structures. (BCT = basal ciliated tract, GD = gill demibranch, LOG = lateral oral groove, MS = mucous string, iPL = inner palp lamella, oPL = outer palp lamella).

and palp, at a region that we call the "marginal gill-palp junction" (Figs. 8B; 9B). Unlike the basal junctions, the location of a marginal gill-palp junction is not fixed. Once the transport of a mucous string is established, the position at which the string leaves the marginal groove can move posteriorly. Mucous strings continually ran from the gills to the palps, even when few particles were carried in the strings. If a mucous string broke, the material in the mar-

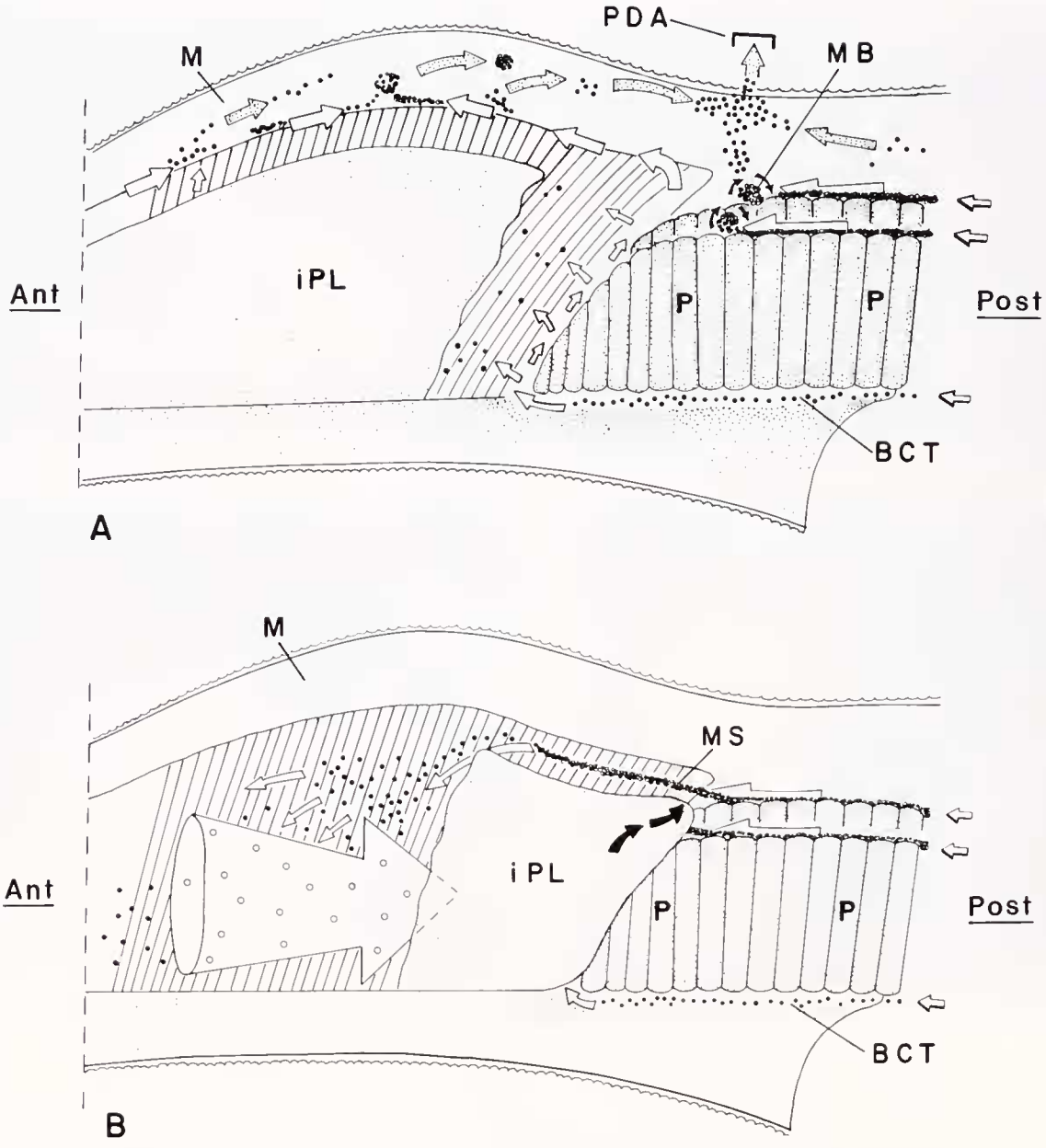


Figure 8. Schematic diagram of the gills and labial palps of *Crassostrea virginica*. The pallial structures are shown in a lateral view, and the inner palp lamella is drawn in cut-away to reveal the ridged surface of the opposing lamella. Arrows indicate direction of net particle movement. (A) The apices of the palps are retracted away from the demibranch margins and mucous balls form at the anterior termination of each marginal ciliated groove. Only particles from the basal tracts are transported onto the labial palps. Mucous balls and excess material from the distal margins of the palps can be transported onto mantle rejection tracts (stippled arrows), carried to the principal discharge area, and then expelled as pseudofeces. (B) The palp apices have contacted the demibranch margins, and particles are transported onto the palps in mucous strings. On the ridged surfaces, the cohesive integrity of the strings is reduced and the entrapped particles disperse (solid circles). When the two opposing palp lamellae spread slightly, some particles are directed posteriorly in an off-surface counter-current (open circles, large circular arrow). After sorting, particles are transported to the lips (smooth region at anterior edge of palp) and then the mouth (not shown) for ingestion. Mucous balls and strings can also be carried across the smooth surfaces of the palps and enter the space between opposing lamellae at the palp apices (solid arrows). (Ant = anterior, BCT = basal ciliated tract, MB = mucous ball, MS = mucous string, P = plica, iPL = inner palp lamella, PDA = principal discharge area, Post = posterior).

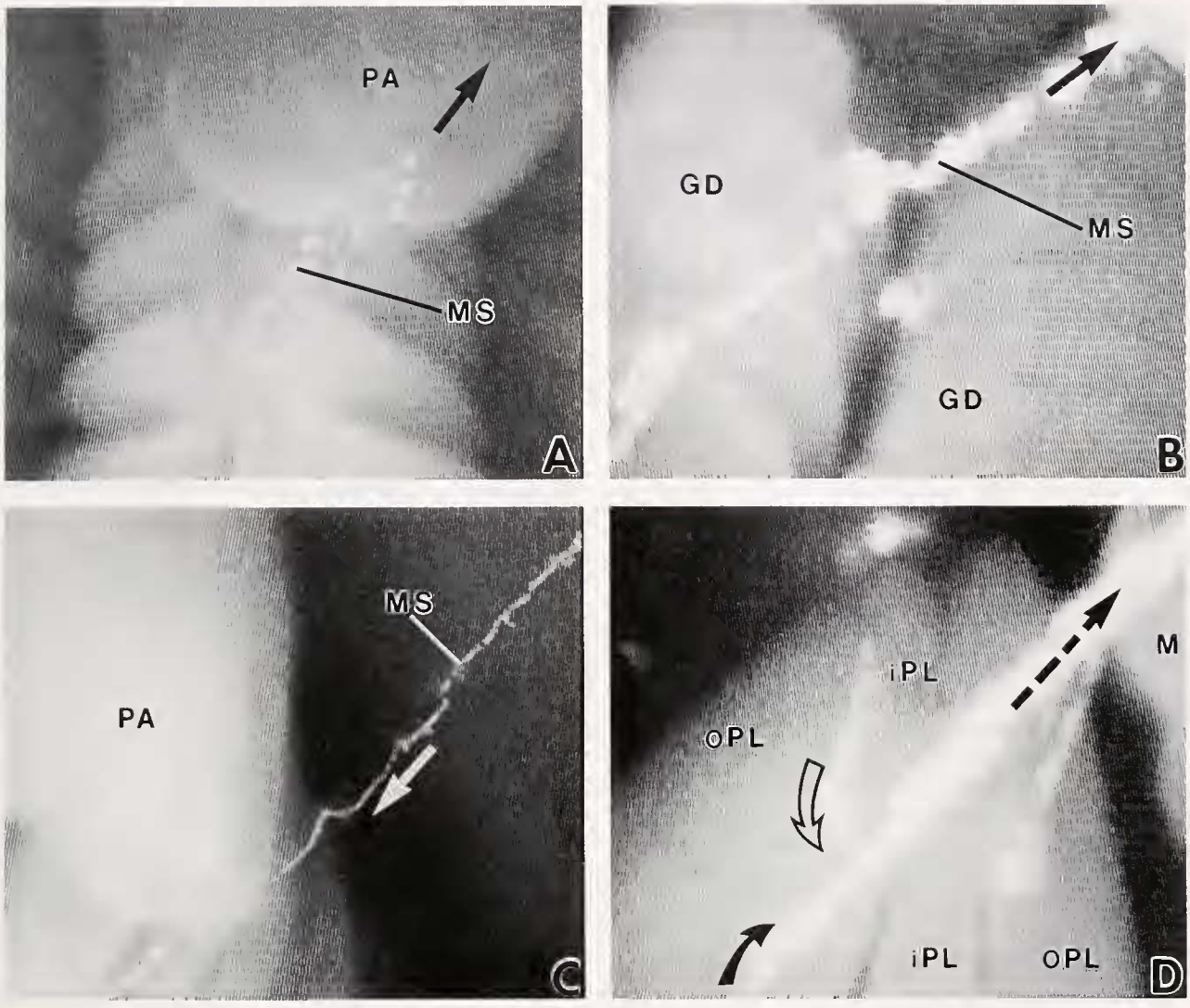


Figure 9. Video-endoscope micrographs showing, *in vivo*, the movement of mucus-bound particles on several pallial structures of *Crassostrea virginica*. Arrows indicate direction of net movement. (A) At the marginal gill-palp junction, the apex of one palp can be seen lifting material from the marginal groove and (B) establishing mucous string transport from demibranch to palp (width of each plica = about 250 μm). (C) At the marginal gill-palp junction, a mucous string is shown bridging the space between two adjacent demibranchs (width of each plica = about 250 μm). (D) Rejection of mucus-bound particles from the distal margins of the labial palps is shown. Material is transported both anteriorly (solid arrow) and posteriorly (open arrow) by cilia on the palp margins. At the junction of the two oppositely beating ciliated tracts, mucus-bound particle aggregations and strings form and are transported (dashed arrow) to mantle rejection tracts to be expelled as pseudofeces (length of palp section = about 4 mm). See Figures 1–4 for descriptions of the form and function of pallial structures. (GD = gill demibranch, M = mantle, MS = mucous string, PA = palp apex, iPL = inner palp lamella, oPL = outer palp lamella).

ginal groove began forming another mucous ball, and the process was repeated.

As described in section A2, the two lamellae of one pair of palps were often observed surrounding only one demibranch. This condition, however, did not prevent material from the unenclosed demibranch from reaching the ridged surfaces of the palps. The particle string from a marginal groove of an unenclosed demibranch could bridge the space between adjacent demibranchs, thereby joining the string moving from the groove of the enclosed demibranch (Fig. 9C). The bridge was established when a mucous ball on the unenclosed demibranch contacted the smooth ciliated surface of the closest palp lamella. The mucous ball, with attached string, was carried to the

apex of the palp, then transported around the free edge and onto the ridged surfaces of the two opposing palp lamellae (Fig. 8B).

Mucus-bound particle strings that were moving from the marginal grooves entered between opposing labial palp lamellae and were directed proximally by cilia on the ridged surfaces (Figs. 2A; 8B). Presumably, this material joined with that introduced from the basal ciliated tracts, which also was directed onto the ridged surfaces. Alternatively, strings could be transported anteriorly along the first two-thirds of the distal margins of the palps to a point close to the fusion of palps and lips (Fig. 8A; 9D). At this location, movement ceased because cilia on the remaining one-third of the margins were beating posteriorly. This arrangement is analogous to that on the margins of the gills. The mucus-bound material on the palp margins was then transferred to the mantle where it was rejected as pseudofeces through the "principal discharge" area (Galtsoff, 1964; Fig. 8A). Our observations on *C. virginica* are similar to those of Yonge (1926), who reported that tracts on the labial palp margins of *Ostrea edulis* beat in opposite directions.

The cohesive integrity of mucous strings from the marginal grooves, as well as any particle aggregations from the basal tracts, was disrupted as the material moved across the ciliated ridges of the labial palps (Figs. 2A; 7D; 8B). Particles were released from the mucous matrix, apparently by the mechanical action of the palps. Particles dispersed across the palps were initially in contact with the ridged surfaces and were transported, in general, towards the lips and mouth, presumably by mucociliary action. Other material, however, was in suspension in the space between each pair of opposing palp lamellae and was directed posteriorly towards the gill-palp junction in intermittent, pulse-like currents (Fig. 8B). These suspended particles appeared to be captured and directed back onto the ridged surfaces of the palps by the smooth ciliated tracts that lie at the free edges of the palp lamellae (Fig. 2A; 8A). Consequently, some of the material between opposing palp lamellae was in the form of a slurry. Occasionally, a portion of this slurry would escape from the space between the palps when the distal margins of the lamellae were not tightly appressed (*e.g.*, Fig. 4B). Material destined for rejection was transported to the distal margins of the palps, then onto the mantle, and rejected as pseudofeces (Fig. 8; 9D).

3. Lips and buccal region. Particles in the buccal region between the inner and outer lips were suspended in a slurry. This slurry, however, appeared more opaque than that between the palps, and the particle aggregations were more numerous. Probably the concentration of particulate matter in the buccal region is higher than that in the basal tracts or between the palps. When we exposed the oyster to a higher particle concentration, the slurry became so thick that the region became difficult to examine and the OIT became covered in mucus.

The slurry in the buccal region was moved towards the mouth by the combined action of the anteriorly beating cilia on the lateral edges of the lips (adjacent to the ridged surfaces of the palps), and the orally beating cilia on the inner lip ridge and outer lip tract (Fig. 5B). Thus, the insertion of the inner lip into the outer lip was very effective in preventing the slurry from escaping from the free margins of the lips. This was in contrast to the labial palp margins, which occasionally allowed particles to escape into the pallial cavity. Insertion of the inner lip into the outer lip also prevented material from entering the buccal region across the free margin of the inner lip, via the central gutter. Such movement of particles across the free margin of the inner lip was reported by Galtsoff (1964, p. 118) from observations of surgically altered oysters, but was never observed by us with the endoscope.

In dissected oysters, particles were not suspended in a slurry. Only a highly cohesive mucus-bound material was observed between the labial palps, lips, and in the buccal region.

4. Esophagus and stomach. Material entering the mouth, esophagus, and stomach of the oyster was in the form of a slurry. This ingested slurry was similar to that found in the buccal region, but appeared to be more opaque, containing numerous small and large particle aggregations. The slurry in the pyloric caecum was slowly swirled, probably by the rotating action of the style. Particle strings were never observed in the alimentary canal, nor were strings ever seen wound around the crystalline style.

Discussion

Many previous reports on the structure and function of the feeding organs in *Crassostrea virginica* were confirmed by our *in vivo*, endoscopic observations. But endoscopy also elucidated several important processes that were not found in literature accounts or were different from those previously reported. Many of these discrepancies could be due to artifacts that were produced when specimens were prepared for prior studies. For example, our endoscopic observations clearly show that particles in the basal tracts, between the labial palps, in the buccal region, as well as in the stomach are transported in the form of a slurry. Previous reports of continuous mucous strings or masses in these regions are incorrect (*e.g.*, Nelson, 1923, 1960; Bernard, 1974; Beninger *et al.*, 1991) and are probably a consequence of dissection which destroys the hydrodynamic properties of the slurry, causing it to collapse into what appears to be mucus-bound par-

ticle-strings, sheets and masses. Similarly, the ciliated tracts on the lips of the oyster have never been described before, probably because the lip-cilia are neurally controlled and cease to function normally after dissection.

Our endoscopic observations of particle capture and transport allow us to reevaluate the previously published concepts of suspension-feeding processes in *C. virginica*. We discuss below our combined observations of organ form and function, providing an integrated view of feeding dynamics in this species. We show, in particular, how our novel observations revise, refine and extend previous models of suspension-feeding mechanisms in *C. virginica*.

Particle capture by the gills

Our observations *in vivo* confirm the complexity of particle capture and are consistent with previous hypotheses concerning this process in bivalves with heterorhabdic gills (*e.g.*, Owen and McCrae, 1976; Owen, 1978; Beninger *et al.*, 1992). In *C. virginica*, particles are captured by the gills in either of two ways: they can be drawn directly into the plical troughs, or they can be captured on the plical crests (Fig. 6). Particles that enter the plical troughs directly are probably entrained by basally directed currents produced by the fine frontal cilia of the principal and transitional filaments. The mechanism of particle capture on the plical crests, however, seems to be more complex.

On the plical crests, particles are immediately captured by the frontal surfaces of ordinary filaments, or they are deflected away from those surfaces and "bounce" to adjacent filaments (Fig. 6). "Bouncing" or "jumping" of particles on the gill lamellae has been described previously in *C. virginica* (Galtsoff, 1964) and in several other bivalve species (Atkins, 1937a; Jørgensen, 1975, 1976; Beninger *et al.*, 1992). The cause of this "bouncing" is not known, but the phenomenon has been attributed to direct contact with cilia or cirri (Atkins, 1937a; Galtsoff, 1964) and to the influence of "local water currents produced by the activity of the metachronally beating band of lateral cilia" (Jørgensen, 1975, p. 216). Unfortunately, the resolution of the endoscope was not sufficient to elucidate the mechanism responsible for particle "bouncing." Jørgensen (1975, 1976) reported that "bouncing" occurred as particles passed between gill filaments and through the interfilamentary spaces, and suggested that particle "bouncing" is a characteristic of "leaky" gills, such as those treated with serotonin. Our observations, however, indicate that particle "bouncing" occurs on the frontal surfaces of filaments, not between filaments, in normal active gills. We suggest that such "bouncing" from one ordinary filament to another maintains particles in suspension above the plical crests and facilitates their deflection into the plical troughs.

After capture, particles are transferred from the laterofrontal cirri to the ciliated tracts on the frontal surface of the ordinary filaments. Ribelin and Collier (1977) suggested that captured particles must be transferred directly to the fine-frontal ciliated tracts, which beat basally, before being moved to the coarse frontal cirri which beat towards the gill margins. Our observations indicate that this sequence does not always occur. Although many particles were observed moving basally first and then reversing direction and moving marginally on the ordinary filaments, other entrained particles immediately moved toward the gill margins. This suggests that other mechanisms, in addition to the direct transfer of particles from the laterofrontal cirri to the fine frontal cilia, are involved in particle capture (*e.g.*, Jørgensen, 1990).

Particle transport by the gills

Previous research on bivalves has shown that the various ciliated tracts of the gill transport particles differently. For example, using isolated heterorhabdic gills of several bivalve species, Jørgensen (1976) noted that particles in the plical troughs move at a faster rate than particles on the plical crests. In juvenile *C. virginica*, Menzel (1955) observed that particles in the marginal grooves move slower than the more discrete particles in the basal tracts, and suggested that this was due to the greater amounts of viscous mucus in the former. Similarly, data from the ciliated surfaces of other organisms indicate that the transport rate of particles decreases as the viscosity of mucus increases (Winet and Blake, 1980; Winet *et al.*, 1982).

Our *in vivo* observations confirm the findings of Menzel (1955) and Jørgensen (1976), and we suggest that such variations in the speed of particle movement reflect functional differences (Table I). The gills of the oyster can be conceptualized as being composed of two distinct transport systems: (1) the plical crest–marginal groove system, and (2) the plical trough–basal tract system. These two systems can be distinguished based on the speed and uniformity at which they transport particles. Such differences reflect the distinctive transport mechanism of each system as explained below, and may be a consequence of anatomical structure (see Ward *et al.*, 1993).

Particles on the plical crests are transported in close association with the frontal surface of the ordinary filaments. These particles move uniformly at a mean velocity of 120 $\mu m \cdot s^{-1}$. In addition, particles on the same ordinary filament can pass in opposite directions as some are directed basally by the fine frontal cilia, while others are directed marginally by the coarse frontal cirri. This complex, bi-directional transport of particles is difficult to explain in hydrodynamic terms, but is characteristic of mucociliary transport. Once transferred to the marginal

groove, particles are bound in a cohesive mucous string. Particles within this string move uniformly and at a velocity ($\bar{X} = 280\ \mu m \cdot s^{-1}$) similar to that of particles on the frontal surface of the ordinary filaments (Table 1). We therefore conclude that mucociliary transport is responsible for moving particulate matter along both the plical crests and the marginal ciliated grooves (Fig. 6).

In contrast, particle movement in the plical troughs and basal ciliated tracts is less uniform than on the plical crest or in the marginal grooves, and particle velocity is 3–5 times greater (Table 1). Because the plical troughs lie deep within the plical folds, we had difficulty determining whether all particles in these troughs are transported in suspension. At the base of the gills, however, these particles exit the plical troughs as individuals or as small aggregations, and are immediately swept anteriorly, in suspension, by currents in the basal ciliated tracts (Fig. 6). We therefore conclude that hydrodynamic transport is primarily responsible for moving particles along both the plical troughs and the basal ciliated tracts. This conclusion is consistent with previous hypotheses of particle transport in these regions (Owen and McCrae, 1976; Jorgensen, 1976; Owen, 1978).

Particle selection on the gills

The role of the gills in particle selection has been debated in the literature for many years (*e.g.*, Nelson, 1923; Yonge, 1926; Atkins, 1937b; Menzel, 1955; Nelson, 1960; Newell and Langdon, 1994). In the oyster, the sorting of particles based on size and other criteria centers around the heterorhabdic nature of the gills and the two ciliated tracts that beat in opposite directions on the frontal surface of the ordinary filaments. According to the current dogma, small or more desirable particles enter the plical troughs and are carried by the principal filaments to the basal ciliated tracts. Larger and less desirable particles are more likely to be captured by the ordinary filaments, where further sorting occurs. On the ordinary filaments, the fine frontal cilia are thought to trap smaller, more nutritious particles and to transport them to the basal tracts, while less nutritious particles are transferred to the coarse frontal cirri and are then transported to the marginal grooves.

Our *in vivo* observations do not fully support this traditional concept. First, all particles delivered to the oysters, regardless of size or treatment, could enter the basal tracts and marginal grooves. Second, all particles on the ordinary filaments, regardless of size or treatment, were ultimately carried to the gill margin by the coarse frontal cirri. We never observed particles entering the basal tracts via the ordinary filaments, as suggested by Atkins (1937c) or by Ribelin and Collier (1977). If size influences selection on the gills, then the requisite size difference must be greater than that used in our study. Menzel (1955), however, also noticed that carmine particles were carried in both the basal tracts and marginal grooves of juvenile oysters, and he concluded that selection does not take place on the gills. Obviously, more detailed endoscopic studies need to be performed, and with a wider range of particle sizes and types, to elucidate the role of the gills in selection.

Particle transfer from gills to labial palps

The basal and marginal gill-palp junctions are more important in feeding than previously recognized. Indeed, the interactions between the gills and palps can control the quantity of material entering the buccal region in response to fluctuations in ambient particle concentrations. For example, during periods of low seston availability, individual particles and small particle aggregations enter the basal gill-palp junctions and are directed onto different regions of the palps for immediate ingestion or further processing and sorting. Various ciliated tracts of the palps, such as the smooth tract on the posterior edge, ensure that particles are evenly distributed across the ridged, sorting surfaces. At the marginal gill-palp junctions, the apices of the palps are usually in contact with the demibranchs and continually accept mucous strings from the marginal grooves. This material is pulled between the opposing palp lamellae for processing (Figs. 2A; 8B).

In contrast, during periods of high seston availability—when the gills are over-loaded or the ingestive capacity is exceeded—excess particles that enter the basal gill-palp junctions are directed onto the marginal ciliated indentations of the demibranchs and carried posteriorly. Posterior transport of this material ceases at the inflection point where the marginal ciliated groove and marginal ciliated indentation meet, and rotating mucous balls form. If the apices of the palps are retracted from the demibranch margins, the rotating mucous balls continue to grow. Eventually, these balls are swept off the margins and onto the mantle by pallial water currents, to be rejected as pseudofeces. Alternatively, mucous balls and strings can be lifted from the demibranchs by the palps and transported anteriorly, along the distal margins, directly to pseudofeces rejection tracts on the mantle. Menzel (1955) and Galtsoff (1964) observed such "food balls" or "masses" at the marginal gill-palp junctions, but concluded erroneously that these were produced by the palps. Furthermore, Galtsoff (1964) indicated that this material was transported posteriorly along the distal margins of the palps to the apices. Our observations clearly indicate that this report is incorrect. Material is transported anteriorly along most of the palp margins, from the apices to points posterior to the fusions with the lips (Fig. 8A).

Many previous reports have suggested that the marginal grooves of the oyster collect material destined for rejection, whereas the basal tracts accept material destined for ingestion (*e.g.*, Ribelin and Collier, 1977). Our observations show that this concept is not completely accurate and is highly dependent on particle concentration. For example, at low particle concentrations, mucous strings from the marginal grooves can be drawn into the labial palps, the entrapped particles dispersed and ingested. At high particle concentrations, when the basal gill-palp junctions become overloaded, particles from the basal tracts can be diverted posteriorly along the marginal indentation, incorporated into mucous balls, and rejected as pseudofeces.

Our findings (1) that the marginal groove effectively terminates at the inflection point of each gill demibranch, and (2) that the cilia of the demibranch beat posteriorly on the marginal indentation and anteriorly in the marginal groove, are particularly important. This anatomical arrangement serves to remove excess particles from the basal gill-palp junctions and prevents mucous strings from reaching the anterior termination of the gills and entering the basal junctions. The prevalence of this condition among the Bivalvia is not known. But Atkins (1937a), working with the protobranch bivalve *Nuculana minuta* Müller, reported that cilia lining the axial grooves of the primitive gills beat anteriorly up to the twelfth pair of leaflets from the anterior end. From that point on, the cilia beat posteriorly.

Particle transport and selection by the labial palps and lips

Material between the labial palps is composed of individual particles and small particle aggregations. As hypothesized by Newell and Jordan (1983), the palps reduce the cohesiveness of mucous strings and aggregations, and disperse the entrapped particles. Sorting and selection of individual particles by the palps is therefore possible. Our observations of the off-surface posterior movements of particles between the labial palps are similar to those of Galtsoff (1964), who described opposing currents along the lateral oral grooves of the palps. In undissected oysters, however, the countercurrent is clearly much stronger and extends from the lateral grooves to the distal margins of the palps. This countercurrent transports particles from the anterior portion of the palps to the smooth ciliated tracts at the posterior edge (Fig. 8B) and probably allows particles to be cycled over the active ridged surfaces several times before being ingested or rejected. Such a recirculative mechanism would increase the efficiency of particle sorting and selection.

Our observations on the motion of the labial palps of *C. virginica* differ slightly from those reported by Bernard (1974) for *C. gigas*. Using a hand-held cystoscope (endoscope), Bernard (1974) observed that each pair of labial palp lamellae of *C. gigas* was "always closely appressed." During the present study, however, we recorded many hours of video showing the opposing lamellae of the palps alternating between close approximation and slight separation (*e.g.*, Fig. 4A, B). As noted by Bernard (1974, p. 7), when the two lamellae are appressed the opposing surfaces can be thought of as "one entity, rather than two separate surfaces, [and] the result of ciliary activity is different." Our observations support this idea, and we suggest that the alternating positions have a functional significance. When the two lamellae are appressed, particles between the palps are in close proximity to both ridged surfaces, and sorting and anteriorward transport occurs. When the lamellae separate, particulate matter moves to occupy the semi-enclosed space between the opposing surfaces, and this suspended slurry is then transported posteriorly for resorting (Fig. 8B). The off-surface flow may be due to the lateral cilia of the gills, which generate the normal feeding currents of the oyster. When the palp lamellae separate slightly, water could be drawn from between the palps towards the gill, carrying the slurry posteriorly.

Food particles enter the buccal region in the form of a slurry. Insertion of the inner lip ridge into the outer lip forms a "hood" over the buccal region and prevents suspended particles from being dispersed. The cilia on the inner lip ridge and outer lip tract direct food orally and further reduce the possibility of particles escaping from the buccal region (Fig. 5B). These findings support the hypothesis of Gilmour (1964), who suggested that the lips of most monomyarians are modified to enclose the proximal oral grooves and to prevent food that is entering the mouth from being dislodged and lost.

Activation and deactivation of cilia on the palp ridges and lips of the oyster was observed many times, *in vivo*, during our study. Such spontaneous inhibition of pallial organ cilia has been reported previously (Menzel, 1955; Nelson, 1960; Galtsoff, 1964), but the behavioral and physiological factors that elicit variation in ciliary activity are not clear. On one occasion we found that ciliary activity on the inner lip ridge and outer lip tract was stimulated by the presence of the diatom *Chaetoceros muelleri*, suggesting that activity in this region is mediated by food material. The activity was perhaps a response to an increase in the number of particles in the pallial cavity or to metabolites produced by the diatoms, but further research is required to elucidate this behavior.

Regardless of the stimulus, the important point is that the cilia on the palp ridges and those on the lips are independently regulated. These findings suggest that the oyster is capable of discontinuous ingestion. Particles can

be removed from suspension and pseudofeces can be produced without ingestion taking place. Furthermore, ciliary activity on the palps continues after dissection, but that on the lips ceases to function normally. Jørgensen (1975) demonstrated that gill cilia of the mussel, *Mytilus edulis*, are under inhibitory nervous control. We suggest that cilia on the lips of *C. virginica* are also under direct neural control.

Particle ingestion

Material in the buccal region and alimentary canal is suspended in a slurry. No continuous mucous strings were ever observed in these regions, and no strings were observed wound around the crystalline style. Such a mechanism has been proposed by previous workers to explain the ingestion of mucus-bound material by bivalves, and was termed the "capstan (or windlass)" model (Orton, 1923; Morton, 1960; Purchon, 1977). Our *in vivo* observations, however, clearly indicate that this mechanism does not operate in *C. virginica*. Rather, a particle slurry is carried through the mouth and into the stomach, probably by the action of cilia that line the alimentary canal.

Our findings are consistent with the chemical nature of the alimentary canal and the mechanical properties of mucus. The pH of the esophagus and stomach of oysters ranges from 5.0 to 6.0 (Yonge, 1926; Galtsoff, 1964), and the isoelectric point and minimum viscosity of mucus falls within this range (Yonge, 1935). The slightly acidic conditions in the stomach, therefore, would keep the viscosity of the mucus-water slurry low and the accompanying particles suspended. In the stomach this slurry is swirled by the rotating style, as was suggested by Yonge (1926) and Nelson (1925). Rotation of material in the stomach probably enhances extracellular digestion and facilitates contact with the food sorting areas where particles are directed into the intestinal groove or into the ducts that lead to the digestive diverticula.

The role of mucus in feeding

We conclude that mucus plays an important role in the feeding process of oysters notwithstanding recent suggestions to the contrary (Jorgensen, 1990). Particle movement and velocity on the ordinary filaments are consistent with mucociliary transport. In the marginal grooves, material is clearly bound in a continuous mucous string, which is important in transporting food material across the marginal gill-palp junction to the labial palps, and in rejecting excess material as pseudofeces. The viscoelastic nature of the mucous string facilitates this process, enabling the string to bend around pallial organs and stretch across spaces between demibranchs while continuing to transport particles (*e.g.*, Fig. 9C).

In the basal tracts, particles are transported suspended in a slurry. But the free suspension of particles does not necessarily mean that mucus is absent. In the heterorhabdic gills of the scallop, *Placopecten magellanicus* (Gmelin), mucocytes that secrete a low viscosity mucus have been identified on the principal filaments and in the dorsal (basal) tracts (Beninger *et al.*, 1993). In addition, mucus has been chemically identified in the slurries that are transported in these tracts (Beninger *et al.*, 1992). In the scallop, therefore, transport along the dorsal (basal) tracts is by hydrodynamic action, but the medium is a mucous slurry. In the oyster, a similar slurry is present between the palps, in the buccal region, and in the stomach. The presence of mucus in these slurries can be inferred from the work of Beninger *et al.* (1991), who identified active mucocytes and mucus in the peribuccal, buccal, and esophageal regions of five species of bivalves, including *C. virginica*.

Our conclusion about the importance of mucus is supported by the rheological properties of this gel. As an amphoteric compound, the viscosity of mucus can change appreciably due to changes in the pH of the medium (Yonge, 1935). In addition, hydration, shear rate, and the type of mucopolysaccharides and concentration of glycoproteins produced by secretory cells can affect the viscosity of mucus (Litt *et al.*, 1977; Mitchell-Heggs, 1977; Silberberg, 1982; Beninger *et al.*, 1993). Changes in viscosity, in turn, can dramatically affect the rheology of mucus, the properties of which can range from Newtonian (hydrodynamic) to non-Newtonian (viscoelastic; *e.g.*, Winet and Blake, 1980). Thus, mucus is an ideal substance in which to transport particles. Variations in the viscosity and cohesiveness of mucus give the oyster maximum flexibility in rejecting, ingesting, and processing particulate matter.

Conclusions

Suspension-feeding in the eastern oyster *C. virginica* is accomplished by the coordination of both mucociliary and hydrodynamic processes. Particles removed from suspension are transported along the gills and to the labial palps in two different forms, mucous strings and slurries. The palps reduce the cohesiveness of mucous strings and particle aggregations, and disperse the entrapped particles. Particulate matter between opposing labial palp lamellae is not bound in cohesive mucus, so sorting and selection of individual particles is possible. Particles in the buccal region and alimentary canal are also suspended in a slurry. Particles can be routed in a variety of directions on the pallial organs and, together with independent control of ciliary activity on the labial palps and lips, allows the oyster maximum flexibility in particle processing, pseudofeces production, and ingestion.

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